Distribution of Anaerobic Bacteria Isolated from the Area around Syowa Station in the Antarctic

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南極昭和基地を中心とした嫌気性菌の分布

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要旨: 南極大陸の昭和基地を中心とした地域の土壌から，193 株の有芽胞偏性嫌気性菌 (clostridia) を分離した．そのうち 155 株を 11 菌種に同定できた．

C. perfringens, C. bifermentans, および *C. sordellii* の順に多数分離された．*C. sporogenes, C. plagarum, C. paraperfringens, C. septicum, C. tertium, C. cadaveris, C. butyricum* および *C. felsineum* は少数分離された．

人や動物によって汚染され難い地域からも clostridia が多く検出された．

南極土壌中の clostridia の分布および特徴を日本の土壌と比較した．

1) 日本の土壌からは検出されてない *C. sordellii* および *C. paraperfringens* を南極土壌から多く分離した．

2) 南極土壌中の菌数分布は好気性菌より嫌気性菌の方が多く，日本の土壌と逆であった．

3) 抗生剤の感受性試験のうち，tetracycline 系に対して南極土壌からの clostridia は，対照菌株に比してより感受性であった．

Abstract: From the soil in the area around Syowa Station, East Ongul Island, Antarctica, 193 strains of clostridia were isolated and identified. It was interesting that many clostridia were contained in the soil samples taken from the places which were considered to be scarcely contaminated by human beings and animals.

One hundred and fifty-five strains were assigned to 11 species, and *C. perfringens, C. bifermentans* and *C. sordellii* were isolated frequently, while *C. sporogenes, C. plagarum, C. paraperfringens, C. septicum, C. tertium, C. cadaveris, C. butyricum* and *C. felsineum* less frequently.

The peculiar distribution and characteristics of the clostridia in the Antarctic soil were discussed in comparison with those found in the soil in Japan.

1) *C. sordellii* and *C. paraperfringens* were never isolated from the soil in Japan.

2) Distribution of aerobes was less extensive than anaerobes in the Antarctic soil.

3) To the tetracycline group among antibiotics tested, the Antarctic strains were more susceptive than the control (Japan) strains.

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1. Introduction

Although aerobes and fungi in the soil of Antarctica were reported (EKELÖF, 1908; DARLING and SIPLE, 1941; SONEDA, 1961; TSUBAKI, 1961; GOTO *et al.*, 1969; RICE *et al.*, 1975), few studies on anaerobes have been performed to date (PRÉVOT, 1948; PRÉVOT and MOUREAU, 1952).

The author, one of the members of the 13th Japanese Antarctic Research Expedition, stayed at the Syowa Station from January 1972 to February 1973 and collected many soil specimens at places within an area of about 300 km around Syowa Station (Fig. 1). Then soil samples were brought back to Japan. Because soil specimens were stored for more than one year before cultivation in this laboratory, non-sporeform-

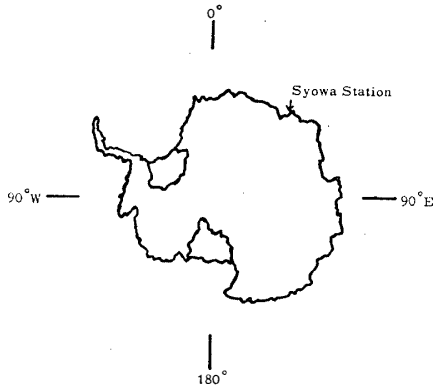

Fig. 1. The location of the Syowa Station (Japan), in Antarctica.

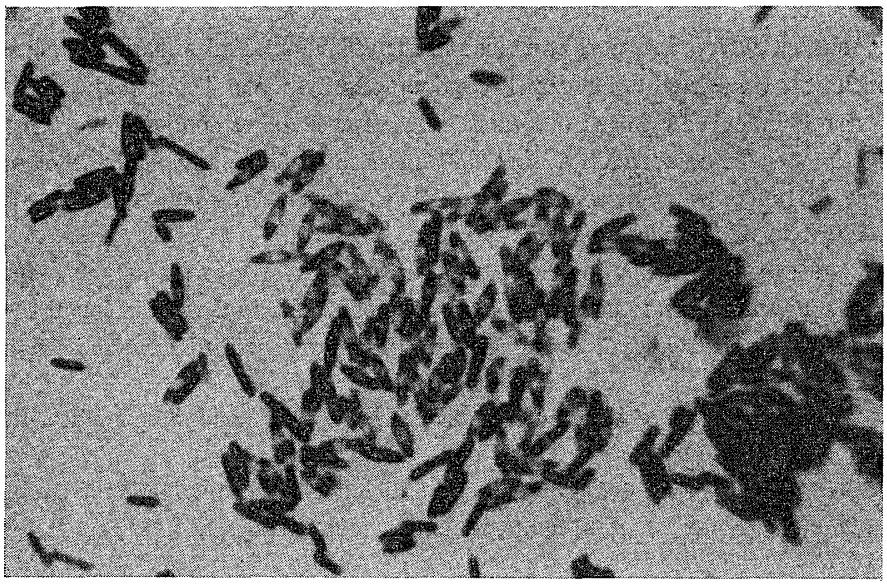

Fig. 2. C. sporogenes (JAM-239) isolated from the Antarctic soil. Gram-stain shows large Gram-positive bacilli, some with subterminal spores. ×5,000. *JAM: Japanese Antarctic Miwa.*

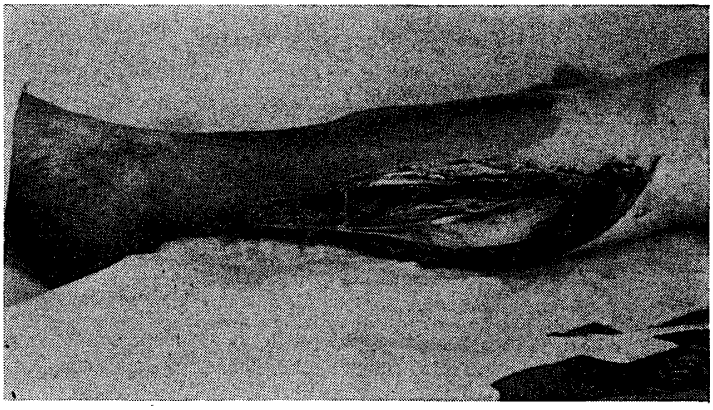

Fig. 3. Leg infected with gas-gangrene by anaerobes.

ing anaerobes were considered to be unable to survive and attention was given only to clostridia.

Clostridia are sporeforming and strictly obligatory anaerobic rods (Fig. 2), and commonly found in soil, marine and fresh water sediments. Some clostridia are known to induce gas-gangrene (Fig. 3), tetanus and sometimes toxic infection.

2. Material and Methods

2. 1. Sampling

Places and procedures for the sampling of soil were given in the previous papers (MIWA *et al.*, 1974; MIWA, 1976).

2. 2. Isolation of clostridia

The soil samples preserved in a frozen condition were thawed at room temperature; about 2 g of a sample was mixed with a basal medium (about 10 m*l*) in a medium-

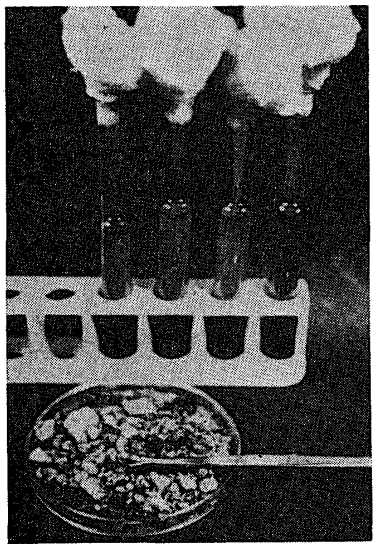

Fig. 4. Each soil samples was mixed with the GAM semisolid high layer medium containing 10% blood.

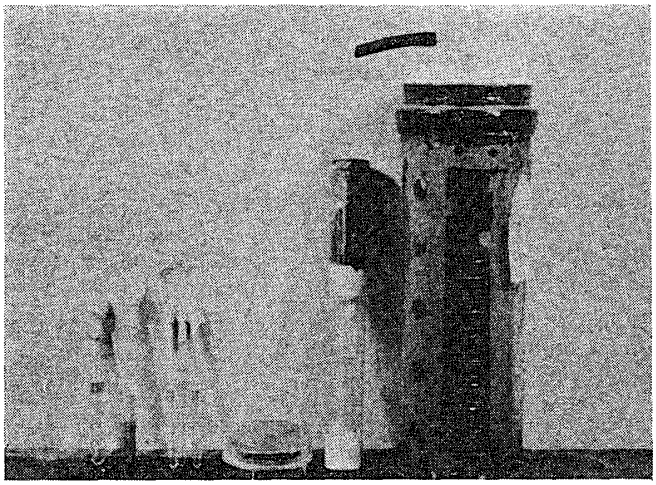

Fig. 5. Anaerobic jar of Gifu University type.

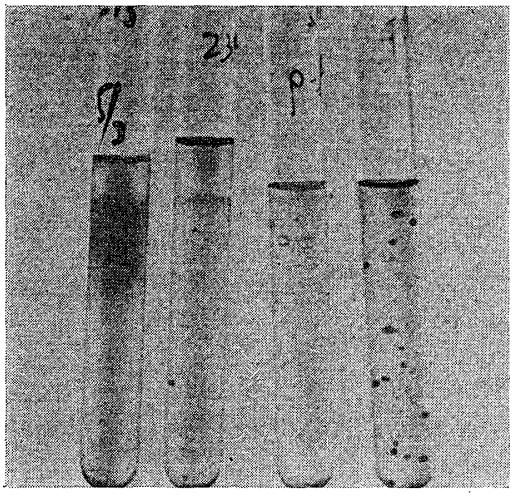

Fig. 6. Cultured anaerobes. Surface layer of the medium dose not grow.

size test tube, and incubated at 37°C for 1–10 days for enrichment. GAM semisolid medium (Nissui) were used as the basal medium (Fig. 4). Clostridia were isolated according to the procedures described by Suzuki's methods (KOSAKAI and SUZUKI, 1968) (Figs. 5 and 6). Anaerobic culture on a plate medium was made by the steel wool method (N_2: CO_2=80%: 20%) (UENO, 1964) at 37°C for 24–48 hours.

2. 3. Identification of clostridia

The author used the technique that was employed in author's laboratory and VPI manual (HOLDEMAN and MOORE, 1972).

2. 4. Quantitative cultivation of soil

The distributions of anaerobes as well as aerobes in the Antarctic soil were examined quantitatively. Simultaneously, soil samples taken in the area around Gifu-city

were examined. One gram of each soil specimen was suspended homogeneously in 9 m*l* of the diluent from which ten-fold serial dilutions were made. A 0.1 m*l* portion of each dilution was spread on each of two GAM agar (Nissui) plates. After incubation at 37°C for 48 hours anaerobically or aerobically, the colonies developed were counted and picked up for purification and further testing. In case of aerobes, only morphology of the colony and Gram staining of the cell were examined (MIWA, 1975a),

2. 5. Susceptibility of *C. perfringens* to antibacterial agents

Twenty-five strains of *C. perfringens* from the Antarctic soil, 37 strains from the area around Gifu-city and one strain from a clinical specimen were examined. As the antibacterial agents, Penicillin-G (PC–G), Cephaloridine (CER), Clindamycin (CLDM), Lincomycin (LCM), Tetracycline (TC), Oxytetracycline (OTC), and Doxycycline (DOTC) were used. Minimum inhibitory concentration (MIC) was examined by Watanabe's method (WATANABE, 1974; MIWA *et al.*, 1975b).

3. Results

As shown in Tables 1 and 2, 193 strains of clostridia were isolated from the Antarctic soil and 155 strains were identified and assigned to 11 species which were the followings; *C. perfringens, C. bifermentans, C. sordellii, C. sporogenes, C. plagarum, C. paraperfringens, C. septicum, C. tertium, C. cadaveris, C. butyricum* and *C. felsineum.*

It was surprising that many varieties of clostridia were found in the soil specimens taken from the places where contamination by human beings, penguins or other animals was hardly possible. The specimens included those from moraine, permafrost and from the bottom of the lake which is covered with thick ice layers throughout the year.

C. perfringens, C. bifermentans and *C. sordellii* (Fig. 7) were the most predominant species; although *C. sporogenes* was isolated less frequently. As for toxigenicity, mostly within 24 hours after intraperitoneal or intramuscular inoculation only *C. perfringens* (Fig. 8) and *C. septicum* killed mice (Figs. 9 and 10). On the other hand, no strain of *C. bifermentans* nor *C. sordellii* was proved to be lethal to mice.

Table 3 shows the distributions of clostridia and aerobes in the Antarctic soil quantitated without enrichment culture and Table 4 shows those in the soil around Gifu-city. Between the above results there are remarkable differences.

Tables 5 and 6 show the values of MIC of several antibacterial agents on *C. perfringens.* The values of MIC of TC, OTC and DOTC against the Antarctic strains were smaller than against the control (Japan) strains.

4. Discussion

The distribution of clostridia in soil has been studied by several investigators

Table 1. Distribution of clostridia

	Sample number	1	2	3	4	5	6	7	8	9	10	11
		East Ongul Island			West Ongul Island			Rumpa Island			Padda Isl.	Ongul-kalven Isl.
Antarctic soil	Places of sampling	Bottom of Lake Mizukumi	Around L. Midori	Bottom of L. Taratine	Bottom of L. Ô-ike	Around L. Higashi	Hill (ground)	Penguins' rookery α (south)	Penguins' rockery (north)	Lake edge (permafrost)β	Lake edge (ground)	Hill (ground)
	Grade of contamination[δ]	A	A	A	A	A	A	A	A	A	C	A
Isolated clostridia	*C. perfringens*	+	+	+		+		+	+	+	+	+
	C. bifermentans	+	+		+	+		+	+			+
	C. sordellii	+	+			+		+	+			+
	C. sporogenes			+								
	C. plagarum		+					+				
	C. paraperfringens								+			
	C. septicum											
	C. tertium						+					
	C. cadaveris				+							
	C. butyricum											
	C. felsineum										+	
	Unidentified strains	+	+	+		+	+	+	+	+	+	+

α rookery: The breeding places of penguins.

β permafrost: Soil ground which is frozen all the year round.

γ moraine: The earth under the thick ice layer (2,500 to 3,000-m depth) in the central area of the Antarctica is being transferred very slowly (15–20 m per year) by the glacier down to the coast. During the slow movement soil and stones had been pushed out toward the surface of the glacier and at last were deposited at the coastline. They are called moraine.

(ZEISSLER, 1930; SASAKI, 1934; PRÉVOT, 1948; NOTOMI, 1957; TAKAGI, 1960). Their findings seem to be in good agreement in numerical ratios of isolates of each species; *C. perfringens, C. sporogenes* and *C. bifermentans* were most frequently isolated and *C. sordellii* very rarely. Similar results were obtained in the author's examination of the soil taken around Gifu-city. However, the present study of the Antarctic soil revealed quite a different pattern in the clostridial distribution. The peculiarities of the Antarctic soil were as follows:

isolated from the Antarctic soil.

12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Continental coast			Langhovde*										Skarvsnes*				Skallen*		
Cape Hinode	F0* point (moraine)$^{\gamma}$	F0 point (permafrost)	Bottom of L. Yuki	Around L. Yuki	Around Yukidori Valley	Bottom of Kami-kama	Hill (ground)	Velow* (mosses)**	Velow (ground)	Velow (moraine)	Hamna* (moraine)	Langhovde (moraine)	Around L. Suribati	Bottom of L. Kizahasi	Around L. Kaminoike	Hill (permafrost)	Bottom of L. Õike	Bottom of L. Dairi	Coast (permafrost)
B	B	A	B	B	A	C	A	B	B	C	C	C	B	C	B	C	B	C	B
+		+	+		+		+	+	+	+	+		+		+				+
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*: Proper names of places.

**: The soil under the ground where mosses grew.

δ A: Contact with human beings and animals was frequent; high grade of contamination.

B: Contact with human beings and animals was infrequent; middle grade of contamination.

C: Almost no contact with human beings or animals; almost no contamination.

+: Positive isolation from one or more of 10 portions of a soil specimen.

(1) *C. sporogenes* was isolated only occasionally; (2) *C. sordellii*, which has never been found in the soil in Japan, was isolated very frequently; (3) *C. paraperfringens*, which has not been found in the soil in Japan, was isolated (4 strains); (4) Although *C. perfringens* was the most predominant inhabitant of soil in both Antarctica and other continents including Japan, the values of MIC of tetracyclines group showed differences between them.

These findings do not support the hypothesis (DARLING and SIPLE, 1941) that the

Table 2. Clostridia isolated from the Antarctic soil (after enrichment culture).

Species	Number of strains	Toxigenicity
C. perfringens	50	+
C. bifermentans	44	−
C. sordellii	41	−
C. sporogenes	6	−
C. plagarum	5	−
C. paraperfringens	4	−
C. septicum	1	+
C. tertium	1	−
C. cadaveris	1	−
C. butyricum	1	−
C. felsineum	1	−
Unidentified strains	38	−
Total	193	

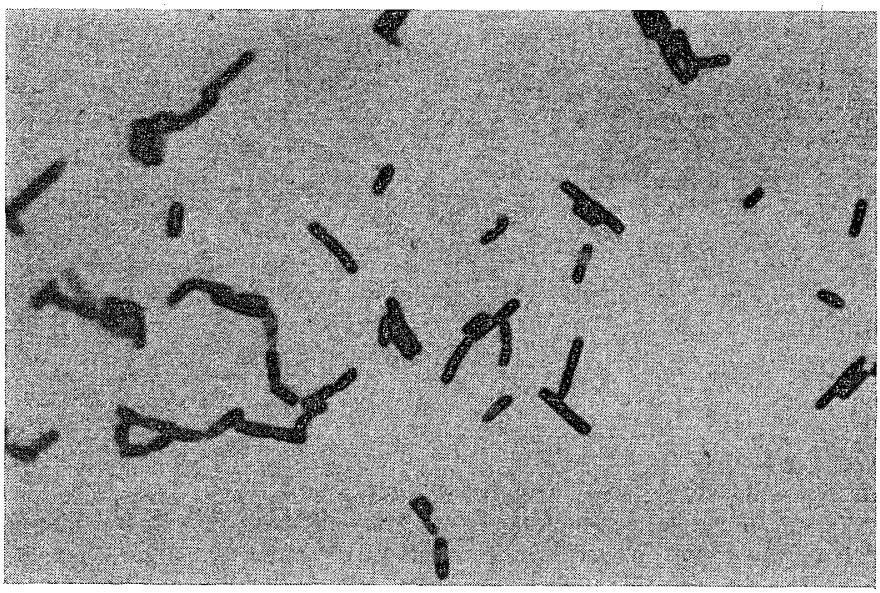


Fig. 7. C. sordellii (JAM-20) isolated from the Antarctic soil. It could not be isolated from the soil in Japan. ×5,000.

origins of sporulating bacteria in Antarctica were transported by air currents from other continents.

It was surprising that many clostridia were isolated from the soil samples taken at the places where almost no contamination by human being or animals was possible (moraine, permafrost and bottom mud of lakes).

Although little attention was paid to aerobes in the Antarctic soil, sporeforming aerobes were about one-third of clostridia when the colonies developed on isolating

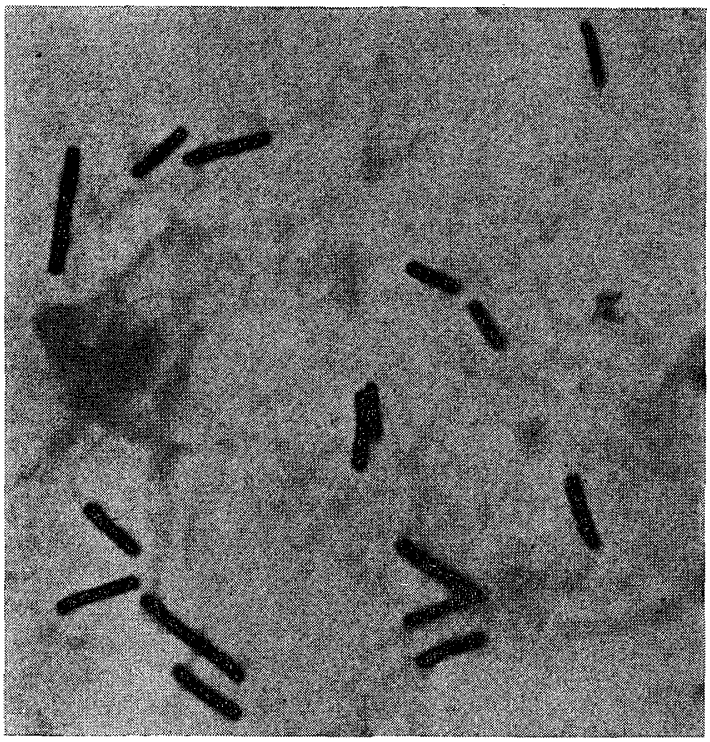


Fig. 8. C. perfringens (JAM-314a) that causes infection of gas-gangrene. ×5,000.

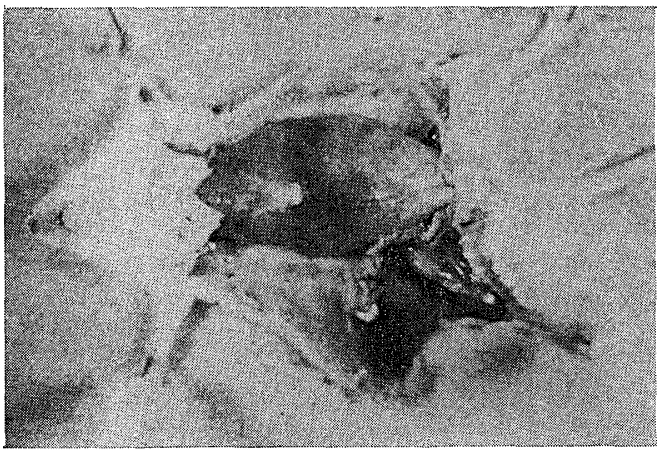


Fig. 9. Most of mice died of gas-gangrene within 24 hours after intramuscular inoculation (r-leg).

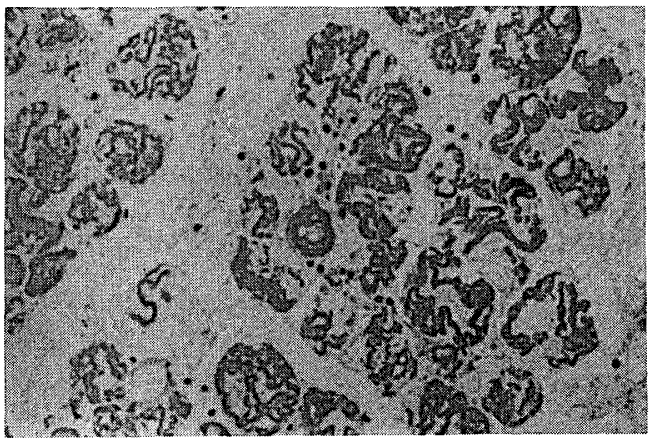


Fig. 10. Autolysis and necrosis of muscle fibers.

Table 3. Quantitative distribution of clostridia and aerobes in the Antarctic soil (without enrichment culture).

Species		Positive isolation/11 specimens	Viable cell counts/g
Clostridia	*C. perfringens*	8*	10^{4-5}
	C. bifermentans	6	10^{2-4}
	C. sordellii	5	10^{2-4}
	C. sporogenes	1	10^{2}
	C. plagarum	1	10^{2}
	C. paraperfringens	1	10^{3}
	C. cadaveris	1	10^{1}
	C. butyricum	1	10^{1}
	C. felsineum	1	10^{2}
	Unidentified strains	10	—
Aerobes**	3–5 species	4	10^{2-5}
	2	3	10^{2-5}
	1	2	10^{2-3}
	0	2	—

*: 11 soil specimens were examined and *C. perfringnes* was isolated from 8 specimens.
**: Species of aerobes were discriminated only by morphology of colonies and Gram-staining of cells.

Table 4. Quantitative distribution of anaerobes and aerobes in the soil around Gifu-city (without enrichment culture).

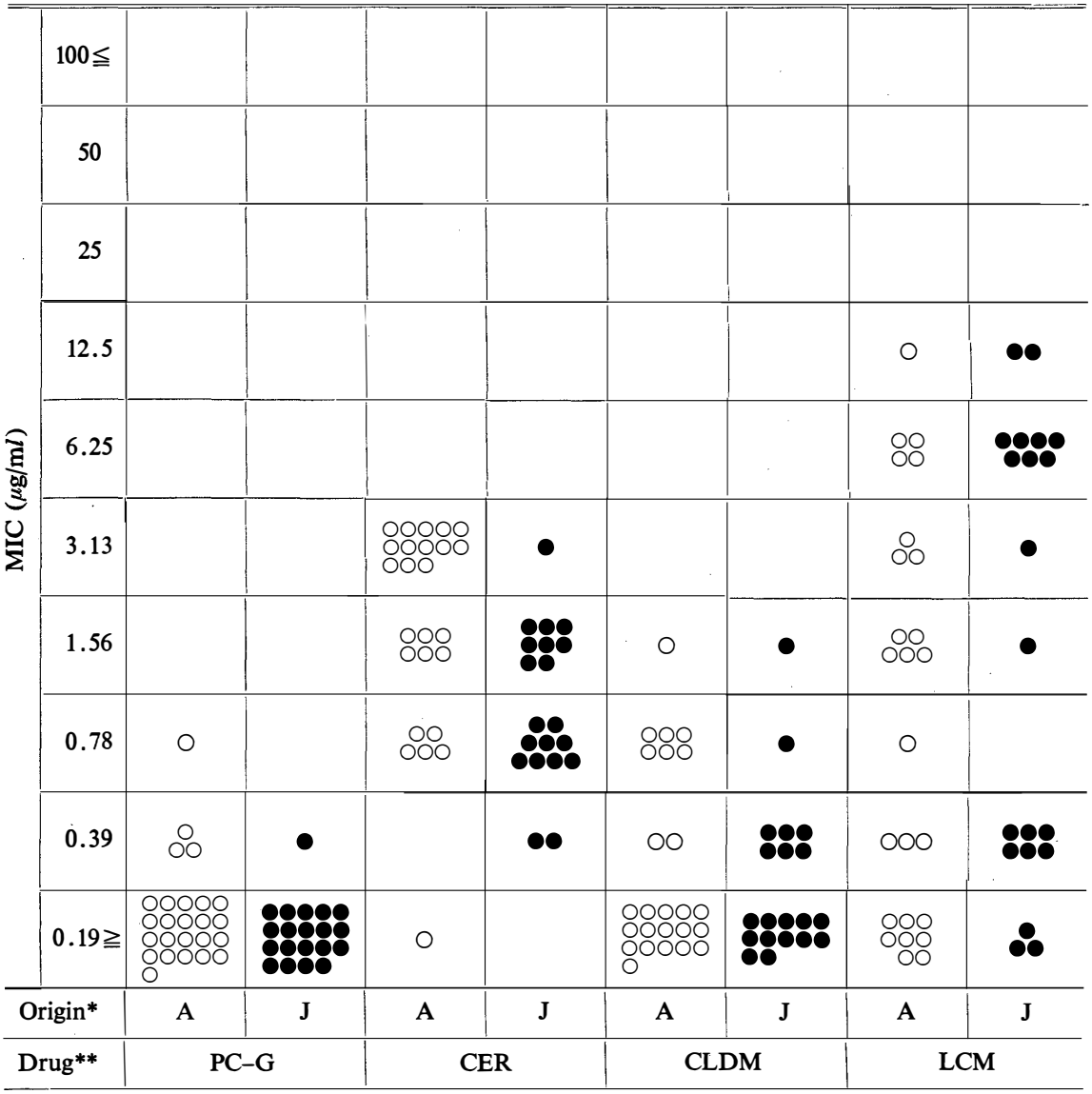
Species		Positive isolation/4 specimens	Viable cell counts/g
Anaerobes	*C. perfringens*	4	10^{3-6}
	C. bifermentans	3	10^{2-4}
	C. sporogenes	3	10^{2-3}
	Other strains*	4	10^{3-6}
Aerobes	6–10 species	4	$>10^{7}$

*: Included non-sporeforming anaerobes.

plates were checked for the strict anaerobiosis.

PRÉVOT and MOUREAU (1952) reported that the anaerobes in the French Antarctic soil specimens were not so different from those found in soil specimens from other continents. However, the numbers of their soil specimens were only eight. Moreover, no strain of *C. bifermentans* nor *C. sordellii* was isolated; the most predominant species were *C. caproicum, C. perfringens* and *C. sporogenes.* There must be, therefore, many factors for the discrepancies between PRÉVOT and MOUREAU and the author's results.

Table 5. MIC of antibiotics on C. perfringens isolated from the soil in the Antarctica and Japan (1).



* A: Antarctic strains. J: Control strains (from soil in Japan).

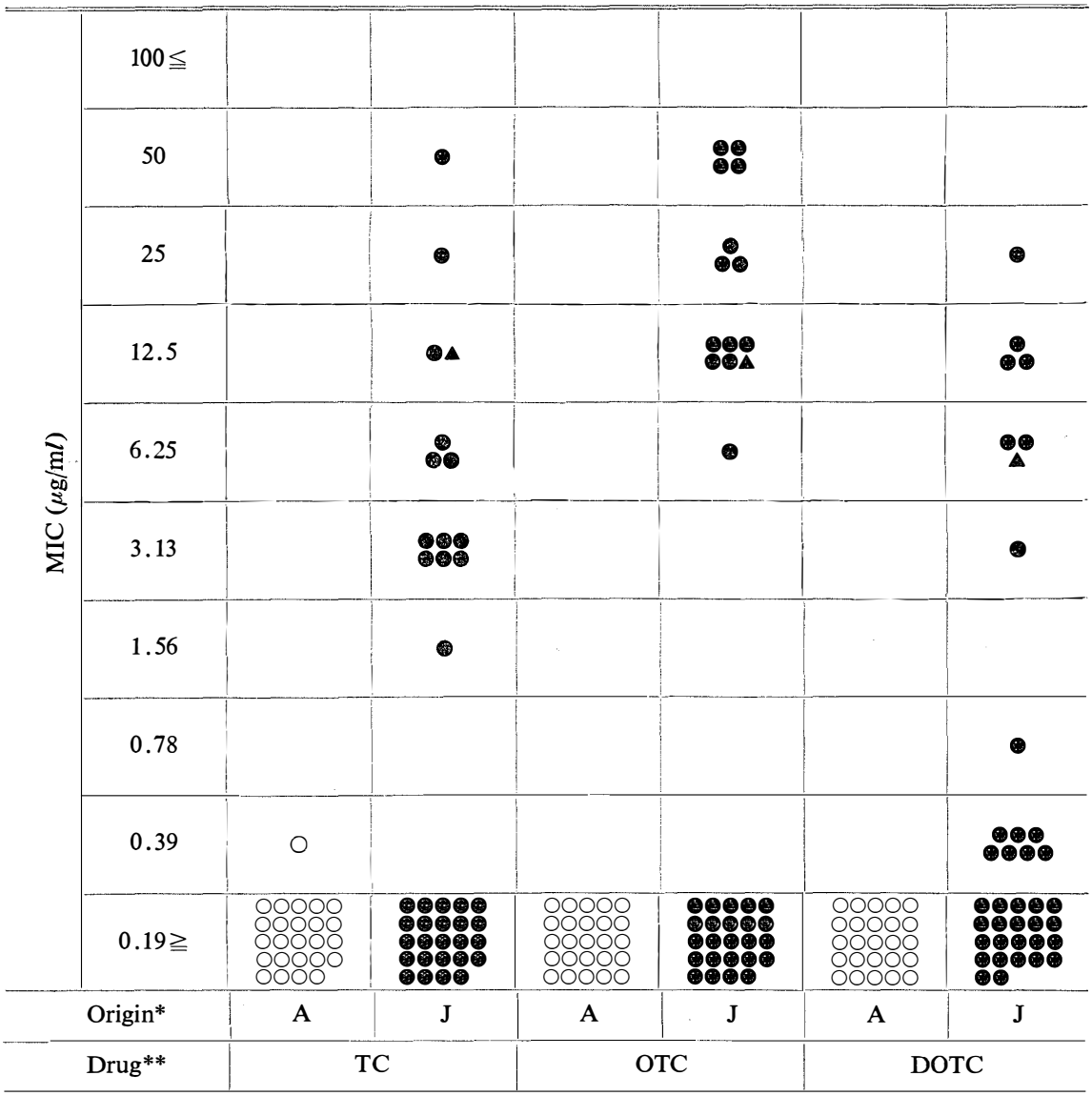
** PC-G: Penicillin-G. CER: Cephaloridine. CLDM: Clindamycin. LCM: Lincomycin.

Acknowledgments

The author is indebted to Prof. S. SUZUKI and Assis. Prof. K. UENO of this laboratory for their valuable advice. Appreciation is expressed also to Prof. H. YOSHIMURA of Hyogo Medical College, Dr. K. IMANISHI of former president of Gifu University, Mr. S. KAWAGUCHI and other members of the 13th Japanese Antarctic Research Expedition for this study.

A part of this work was presented at 47th and 48th Japanese Society Microbiology,

Table 6. MIC of antibiotics on C. perfringens isolated from the soil in the Antarctica and Japan (2).



* A: Antarctic strains. J: Control strains (from soil in Japan).
▲: One strain from clinical specimen.
** TC: Tetracycline. OTC: Oxytetracycline. DOTC: Doxycycline.

22th Japanese Society Chemotherapy and 9th International Society Chemotherapy.

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